Isolation and molecular characterization of *Candida africana* from Jos, Nigeria

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During a survey of the prevalence of *Candida* spp. in Jos, Plateau State, Nigeria, two atypical *C. albicans* isolates were recovered. These two yeasts were germ tube positive, chlamydospore-negative and gave a green color on CHROMagar *Candida*. Molecular analysis performed by amplification of the *hwp1* gene showed that these two isolates belonged to *C. africana*, a newly proposed *Candida* species closely related to *C. albicans*. Based on the presence or absence of an intron in DNA sequences encoding rRNA, the two *C. africana*, including all *C. albicans* isolates examined, were found to belong to genotype A and no other genotypes or species such as *C. dubliniensis* were found. To our knowledge, this is the first isolation of *C. africana* in Nigeria.

**Keywords** *Candida africana*, *Candida albicans*, *Candida dubliniensis*, *hwp1* gene, chlamydospore-negative

Introduction

The genus *Candida* comprises more than 300 species [1] of which over 40 are pathogens [2] and have been associated with life-threatening infections in humans, especially those with an impaired immune system. However, in recent years, the taxonomy of the most important *Candida* species such as *Candida albicans*, *C. parapsilosis* and *C. glabrata* has undergone significant changes due to the description of new closely related species and therefore they are, nowadays, recognized as ‘cryptic species complexes’ [3–6].

In 1995, a group of Irish researchers described for the first time a new pathogenic species called *Candida dubliniensis* which shares several phenotypic and genotypic characteristics with *C. albicans* and it is easily misidentified as such [3]. Six years later, 14 African *C. albicans* isolates with unusual phenotypes and two others from two German patients were proposed as representative of a new *Candida* species called *Candida africana* [7].

Phenotypically, *C. africana* resembles *C. albicans* and/or *C. dubliniensis* although some morphological and biochemical characteristics are clearly different. In fact, *C. africana* isolates form germ tubes in serum but fail to produce chlamydocpores on corn meal agar (CMA) and they are also unable to assimilate N-acetylglucosamine, glucosamine, trehalose and DL-lactate [7]. Nevertheless, on the basis of genetic evidence, *C. africana* cannot be treated as a separate species from *C. albicans* even if it represents the most evolutionary divergent type so far described with a marked propensity to cause mainly vaginal candidiasis [8,9].

During a study to investigate the prevalence of *Candida* spp. in a hospital in Jos, Plateau State, Nigeria, we isolated *C. africana* from two women with suspected vulvovaginitis.

To our knowledge, this is the first report describing the recovery of *C. africana* from Nigeria.

Materials and methods

A total of 320 women presenting symptoms of vulvovaginal inflammation were screened for the presence of *Candida* spp. A total of 177 yeast isolates were recovered
and were initially phenotypically characterized using conventional methods such as the germ tube test in serum at 37°C for 2–3 h, chlamydospore production on CMA at 25°C for 5 d and appearance of the colonies grown on the chromogenic medium CHROMagar Candida (CHROMagar, Paris, France).

Definitive species identification and discrimination of all members of the C. albicans species complex was performed by the amplification of the hyphal wall protein 1 (hwp1) gene as described by Romeo and Criseo. [10]. Further genetic characterization of Candida isolates was carried out according to the ABC typing method described by McCullough et al. [11] which allows us to identify C. albicans genotypes on the basis of the presence or absence of an intron in ribosomal DNA.

**Results**

Among 177 Candida isolates obtained, 84 were presumptively identified as being C. albicans by phenotypic methods. All these isolates, in fact, grew as light-green colonies on CHROMagar Candida and formed germ tubes in serum at 37°C after 2 h of incubation. In addition, for all but two isolates, chlamydospores were also seen in CMA cultures after 5 d incubation at 25°C.

The use of hwp1 gene amplification confirmed the identity of the two chlamydospore-negative isolates as C. africana (Fig. 1A) whereas the remaining 82 isolates were all identified as C. albicans. No C. dubliniensis isolates were found in this study.

Furthermore, an amplicon of approximately 450 bp was obtained by ABC typing [11] indicating that all our C. albicans isolates, including the two C. africana belonged to genotype A (Fig. 1B). In vitro antifungal susceptibility testing, carried out using Sensititre YeastOne Y08 system (Trek Diagnostic Systems Ltd, East Grinstead, UK), showed that our C. africana isolates were or appeared to be susceptible to amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, ketoconazole, voriconazole, posaconazole and caspofungin.

**Discussion**

In recent years, many epidemiological studies of candidiasis have reported the relative prevalence of the common Candida species and C. albicans remains the most frequently isolated species in the world [12]. However, differences emerge when particular groups of patients or specific geographical locations are examined [2]. In particular, C. dubliniensis has been frequently recovered from the oral cavity of HIV-positive and AIDS individuals [13] whereas C. africana have been mainly reported as the cause of vaginitis from several geographical areas [8]. Nevertheless, although the distribution of this pathogenic yeast appears to be worldwide, its real incidence and role in human candidiasis is, currently, poorly understood. Clinically, it seems that there is no clear reason to discriminate C. africana or C. dubliniensis [14] from C. albicans because most isolates of these species are susceptible to common antifungal agents [8,14].

In Europe, C. africana has been isolated from German, Italian, Spanish and British patients [8] but it has not been found in Turkey where a number of vaginal C. albicans isolates have been recently re-examined in an attempt to reveal the cryptic presence of C. africana and/or C. dubliniensis [15].

As evidenced by ABC typing [11], all C. africana isolates recovered so far [8,16], including those examined here, belonged to the genotype A that represents the most encountered and prevalent infectious type of C. albicans found in recent studies [16].

In Africa, most C. africana isolates were recovered from patients in Madagascar and Angola [7] and there are no previous studies reporting its isolation from Nigeria where, indeed, it is well documented that C. albicans is the most common species that causes vaginal infections [17,18]. Therefore, according to the data reported here, it is reasonable to believe that the incidence of C. albicans in Nigeria could be slightly overestimated due to cryptic presence of C. africana.

In this study, no C. dubliniensis isolates were recovered among our clinical samples. This may be due, in part, to...
the type of biological samples (vaginal) examined here, in which *C. dubliniensis* has, generally, a low incidence [19] but this species was also not found in oral cavities of Nigerian patients [20] that represent the most affected body sites by *C. dubliniensis* [13]. In addition, existing epidemiological data indicated that there are pronounced differences about occurrence of *C. dubliniensis* among healthy or HIV-infected African individuals [21] and, in general, this species seems to be more locally or regionally prevalent having been found mainly in South Africa, Egypt and Tunisia [22–24] but not in some other regions of the continent, including Nigeria [20,25,26].

On the other hand, *C. africana* isolates make up only a very small percentage of the *C. albicans* clinical isolates even if, to date, there have been very few studies looking at the prevalence of this yeast among the *C. albicans* species complex [8]. Therefore, further epidemiological investigations are needed to reveal the extent of *C. africana* and/or *C. dubliniensis* prevalence in clinical samples, especially those of African origin. Such studies might detect a different reality than that currently known regarding the epidemiology of these *Candida* species.

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**References**


